REMARKS

Any fees that may be due in connection with the filing of this paper, or during the entire pendency of this application, may be charged to Deposit Account No. 06-1050. If a Petition for Extension of time is needed, this paper is to be considered such Petition.

Claims 1-7, 9-28, 30-50, 53-67 and 79-89 are pending in this application. Claims 12-14, 19-27, 39-50, 56-67, 80, and 82-89, which withdrawn from consideration as non-elected, are retained as the linked by pending claims. Upon allowance of any linking claims, the restriction requirement as to any linked claims will be withdrawn.

Claims 1, 6, 28, 30-33, 53, 55, 57, 59, 61, 63 and 65-67 are amended and claims 8, 29, 51 and 52 are cancelled without prejudice or disclaimer. The claims are amended for clarity. Claim For example, claims 1 and 28 are amended to recite that the is-Hits are identified *in silico*, and claims 1, 28 and 51, and claims dependent thereon, are amended to consistently recite that the is-HIT is locus. Amendment of claims to recite that "a restricted subset is a group of selected amino acids selected to have a predetermined effect on protein activity" finds basis on page 4, second paragraph. Amendment of the claims necessitated cancellation of claims 51 and 52 as duplicative, which further necessitated amendment of claims 53, 55, 57, 59 and 65-67 to properly depend from a pending claim. No new matter is added, nor do the amendments change the scope of the claims.

OBJECTION TO CLAIMS 1, 52 AND 55

Claims 1, 52 and 55 are objected to for minor typographical errors. The amendments of claim 1, cancellation of claim 52, and change in status identifier of claim 55 obviate these minor objections.

THE REJECTION OF CLAIMS 1-11, 15-18, 28-38, 51-55 AND 79-81 ARE REJECTED UNDER 35 U. S. C. 112, SECOND PARAGRAPH

Claims 1-11, 15-18, 28-38, 51-55 and 79-81 are rejected under 35 U. S. C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter that applicant regards as the invention for reasons enumerated and discussed below. Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks.

a. Claim 1 is rejected as failing to provide proper antecedent basis for "the evolved predetermined property or activity" in line 4 because line 2 recites "predetermined property or

activity" rendering the antecedent for "the evolved predetermined property or activity." Amendment of claim 1 to delete "evolved" in line 4 obviates this alleged ambiguity.

b. Claim 1, as well as claims 28 and 51, are alleged to be indefinite because it allegedly is unclear whether an is-HIT is an amino acid or a locus because line 5 of claim 1 states, "wherein each target amino acid is designated as in silico-HIT (is-HIT), " line 4 of step c of claim 1 states, "each replacement is at the same is-HIT locus." Claim 1, line 5 is amended to recite "wherein each target amino acid locus," thereby obviating this rejection. Claims 28 and dependent claims are similarly amended. Claim 51 does not recite this language. Claims dependent on claim 51 that do recite the language are amended to delete it as extraneous.

c. Claim 1 further is alleged to be indefinite in the recitation of "single amino acid replacement " occurs several times without referencing the first recitation as an antecedent. Claim 1 is amended herein to delete redundant language and clarify antecedent basis.

d. Claim 1 further is alleged to be indefinite because in

> step a, the last phrase of the claim states, "each target amino acid is designated an in silica-HIT (is-HIT). " However, in step b, the first line states, "identifying one or more replacement amino acids, specific for each is-HIT. " In this instance, it is unclear for 'claims 1, 28, and 51 if each is-HIT is a single amino acid replacement as stated in step a, or possibly a plurality of replacements as indicated in step b.

As discussed above, as amended, the claims recite that the is-HIT refers to the locus. A plurality of different amino acids can be separately tested at locus to identify candidate LEADs, which re the target protein with a single amino acid substitution.

e. The Examiner further states that recitation of "sets" with reference to the encoded proteins is unclear. The Examiner states:

> if each LEAD protein in a set contains the same amino acid replacement, it is unclear as to if and how each member in a single set of LEAD proteins differs in composition from other members in the same set. Likewise, the same rejection applies to the definition of "set of candidate super-LEAD " proteins in claims 6 and 33."

The basis for this rejection is unclear. Each protein in a set is the same molecule. The term set is used because while individual mutations are introduced one at time into the target proteins, more than one molecule is tested at time. One ends up with a test tube or well or beaker or other vessel containing a plurality of the molecules that are the same.

The claim states "each nucleic acid molecule in a set encodes the same candidate LEAD protein that differs by one amino acid from the target protein or peptide, whereby the encoded candidate LEAD proteins in each set differ by one amino acid from thee encoded candidate LEAD proteins in each of the other sets." They are the same molecules – set is use to render it clear that we are not contemplating a single molecule.

f. The claim also is rejected for reciting in step c that "each replacement is at the same is-HIT locus, whereby each candidate LEAD protein is the same." Step c has been amended to delete this language.

The Examiner states that for purposes of examination, the claims will be interpreted in light specification such that is-HIT is an amino acid locus; a LEAD protein is assumed to be the wild type protein with a single mutation. Each set of LEADS proteins will be assumed to have identical members of LEAD proteins with an identical mutation. The Examiner has correctly interpreted that claims. The amendments herein should comport with this interpretation and the specification as originally filed.

THE REJECTION OF CLAIMS 1, 4-11, 38-35, 51, 52 AND 80 UNDER 35 U.S.C. §103(a)

Claims 1, 4-11, 28-35, 51, 52 and 80 are rejected under 35 U. S. C. 103(a) as unpatentable over Ladner *et al.*, (U.S. Patent No. 5,096,815) in view of Stabach *et al.* (Biochemistry, 1997, volume 26, pages 57-651) because Ladner *et al.* states in its abstract that "DNA-binding proteins, especially repressors of gene expression, are obtained by **variegation** of genes encoding known binding protein and selection for protein binding the desired target DNA sequence;" computational modeling aspect of the sites for mutagenesis on the proteins are described in the Graphical and Computational Tools section in column 62, line 56 to column 63, line 11 and column 63, lines 25-34, which state:

> The most appropriate method of picking the residues of the protein chain at which the amino acids should be varied is by viewing with interactive computer graphics a model of the initial DBP complexed with operator DNA. This model need not come from an X-ray ray [sic]structure of the complex, but could instead be obtained by docking a 3D structure of the initial DBP to an appropriate model of the DNA operator sequence. A model based on X-ray data from the DNA protein complex is preferred. A stick figure representation of molecules is preferred. A suitable set of hardware is an Evans &Sutherland PS390 graphics terminal . . . and a MicroVAX II supermicro computer... The computer model should preferably have at least 150 megabytes of disk storage, so that the Brookhaven Protein Data Bank can be kept on line. A FORTRAN compiler, or some equally good higher-level language processor is preferred for program development... ;

and Ladner *et al.*, states at column 63, lines 25-34 of Ladner *et al.*, that one can use theoretical calculations, assess the effect of a particular amino acid substitution. The Examiner urges that empirical methods are described in column 12, lines 50-79, of Ladner *et al.* which state:

> This application uses the term 'variegated DNA' to refer to ***a population of molecules that have the same base sequence through most of their length, but that vary at a number of defined loci...*** When plasmids containing the variegated DNA are used to transform bacteria, each cell makes a version of the original protein. Each colony of bacteria produces a different version from most other colonies. If the variegations of the DNA are concentrated at loci that code on expression for residues known to be on the surface of the protein or in the loops, a population of genes will be generated that code on expression for a population of proteins, many members of which will fold roughly the same 30 structure as the parental protein. Most often we generate mutations that are concentrated within the codons for residues thought to make contact with the DNA.

Empirical methods and assays (which further define a restricted set of "focused" mutations)are further described in columns 77, line 66 to column 78, line 5 of Ladner *et al.* which states:

> The initial set of 5 residues for Focused Mutagenesis contains residues in or near the N-terminal half of alpha helix 3: Y26, Q27g S28, N31, and K32. Varying these 5 residues through all 20 amino acids produces 3.2 x 10 different protein sequences encoded by 32 . . . different DNA sequences. Since all 5 residues are in the same interaction set, the variegation scheme produces the maximum number of different surfaces.

Ladner *et al.* continues in column 78, lines 17-30 stating:

> We synthesize DNA inserts having approximate level of variegation, ligate the synthetic DNA into appropriately digested and purified plasmid DNA, transform competent cells, select for uptake of plasmid, introduce the cells for production of potential DBPs [DNA Binding Proteins], and then select for the wanted DNA binding phenotype using the binding marker genes. Surviving colonies are screened genetically and biochemically to verify that a novel DBP is effectively binding to the target DNA sequence. DBP characterization includes, at the genetic level, deleting and backcrossing the DBP gene at the protein level, in vitro binding assays; and, at the DNA level, determination of the complete sequence of the DBP gene.

The Examiner concludes that Ladner *et al.*, thus teaches:

a method for generating a protein or peptide molecule, where target amino acids are identified by computer, and candidate substitutions are identified. A collection of mutants with desired properties a produced. They are expressed by genes in plasmids in host bacteria, and they are finally screened. This is a process of site-specific mutagenesis at the restricted sited of 5 specific residues. LEAD (single mutation)and super-LEAD (multiple mutation) proteins are produced and screened within the 3.2 x 10" different protein sequences corresponding to all 20 occurring amino acids. Codons of the genes in the plasmids are altered to result in the mutated proteins. The specific number of amino acids investigated was 5. Ladner et al. does not show a protein with exclusively a single amino acid mutated at exclusively

a single is-HIT locus. Additionally, Ladner et al. does not show each species of mutant peptide being screened individually.

The Examiner urges that Stabach *et al.*, teaches a study in which "twenty different amino acids were substituted by site-directed mutagenesis for wildtype Val 175, the penultimate (P2) residue flanking the major calpain cleavage site in αII spectrin" and that the purpose of the study was to determine the consequences of calpain action specifically on human fetal brain αII spectrin cDNA. Determinants of p-calpain sensitivity were explored using recombinant spectrin peptides in which the Val175 residue at the P2 position relative to the site of cleavage was been replaced by each of the other 19 amino acids. Stabach *et al.* effects the single mutation and screened the resulting polypeptides.

The Examiner concludes that:

> [i]t would have been obvious to someone of ordinary skill in the art at the time of the instant invention to modify the combinatorial study of Ladner et al. in view of the single amino acid replacement study of Stabach et al., because, while Ladner et al. generates combinatorial mixtures of LEADS, Stabach et al. has the advantage of individually screening each species of singly mutant protein at an identical locus for the purpose of understanding the implications of calpain action on spectrin.

This rejection is respectfully traversed.

Relevant law

In order to set forth a prima facie case of obviousness under 35 U.S.C. § 103 the combination of the cited references must actually teach or suggest the claimed subject matter. Further, that which is within the capabilities of one of ordinary skill in the art is not synonymous with that which is obvious. Ex parte Gerlach, 212 USPQ 471 (Bd. APP. 1980). Obviousness is tested by "what the combined teachings of the references would have suggested to those of ordinary skill in the art" In re Keller, 642 F.2d 413, 425, 208 USPQ 871, 881 (CCPA 1981), but it cannot be established by combining the teachings of the prior art to produce the claimed subject matter, absent some teaching or suggestion supporting the combination (ACS Hosp. `

The prior art must provide a motivation whereby one of ordinary skill in the art would have been led to do that which the applicant has done. Stratoflex Inc. v. Aeroquip Corp., 713 F.2d 1530, 1535, 218 USPQ 871, 876 (Fed. Cir. 1983). In addition, the mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggests the desirability of the modification. In re Fritch, 23 USPQ

1783 (Fed. Cir. 1992). There must be a reason why a person of ordinary skill in the art would have combined the elements as claimed. KSR v. Teleflex, Inc. 550 US _____ S.Ct. (2007).

"To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" W.L. Gore & Associates, Inc. v. Garlock Inc., 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

The rejected claims

Claim 1 recites that the nucleic acids in the host cells, and ultimately the encoded proteins, comprise an *addressable* array, which means that the identity of each protein at or that comprises each locus is ***a priori*** known. This is a **consequence** of the methods as claimed. In accord with the claimed methods, single amino acid changes are individually introduced and screened. In all of the prior art methods for directed evolution, a plurality of changes can be introduced, and **mixtures** of proteins are produced. As a result, at the end of the process, a protein that is screened and selected, has to be further identified, such as by sequencing.

In addition, the instantly claimed methods are unbiased in that all species and all permutations of sequence changes at the is-HIT loci are represented and tested. Prior art methods that introduce a plurality of changes and/or produce mixtures or nucleic acid molecules and/or encoded proteins, can result in biased products. Where there is a mixture or plurality of changes introduced, conditions can favor one mutation or mutant over another when produced, grown and/or screened. In addition, as noted, because the proteins are produced singly and individually screened, their identity is known. There is no need to do a further identification step.

This method is quite powerful, in that proteins with very few, in fact, single amino acid changes, with striking changes in properties can be identified. The specification, exemplifies application of the methods to interferons. The method has proven to be very powerful. To date the method has been applied to 1000s of proteins, and candidate LEADS have been identified and are being pursued as therapeutics. A company, the assignee of the instant application, was founded and has successfully developed evolved therapeutic proteins employing this method. None of the cited references nor references of record teach or suggest a method in which amino acids are individually and separately replaced, and the variants separately produced and separately produced. To be clear, as claimed, the method is performed with addressable arrays

so that a multitude of proteins are prepared and screened in parallel (in parallel). The method is a departure from prior art methods that produce mixtures of nucleic acid molecules and variants.

The instant application is based on U.S. provisional application Serial No. 60/409,898, filed September 9, 2002. In accord with the duty of candor, the only art of record that describes a method in which individual changes are separately introduced one-by-one, and separately expressed and screened, is described in co-owned U.S. application Serial No. 10/022,249. The subject matter in the co-owned application first published as the corresponding International PCT application No on February 02, 2003. Hence, to the extent the instant claims find basis in the earliest provisional application, which discloses the claimed method in its entirety, the earlier disclosure is only available under 35 U.S.C. §102. The instantly claimed methods differ therefrom, for example, in requiring *in silico* identification of residues, thereby eliminating the first part of the method in the earlier application in which is-HITS are identified empirically as disclosed in the earlier application.

Further, the attached **Declaration of Dr. Vega**, a joint inventor, discussed below, describes the success of these methods. The attached DECLARATION evidences the power of the method in identifying LEAD compounds that exhibit a predetermined property. Identified LEADS, with a predetermined property, exhibit the property with as few as a single amino acid replacement. The power of the method derives from the semi-rational systematic unbiased method in which by virtue of changing amino acids one-by-one and testing them one-by-one, all changes are equally represented and tested. As a result, LEAD proteins, which exhibit a significant change in a property, can be developed contain as few as single amino acid change, and retain therapeutic activity.

Independent claim 1 recites:

A method for generating a protein or peptide molecule, having a predetermined property or activity, by:

> (a) identifying, within a target protein or peptide, one or more target amino acids amenable to providing the predetermined property or activity upon amino acid replacement, wherein:
>
> the identifying of the one or more target amino acids in step a) is conducted *in silico*; and each target amino acid locus is designated an *in silico*-HIT (is-HIT);
>
> (b) identifying replacement amino acids to replace the residue at each is-HIT, wherein:

the replacement amino acids are amenable to providing the evolved predetermined property or activity to the target protein upon amino acid replacement; and

the replacement amino acids comprise all of the 19 remaining non-native amino acids or a restricted subset of amino acids up to all 19 remaining amino acid; and

a restricted subset is a group of selected amino acids selected to have a predetermined effect on protein activity;

(c) producing a collection of sets of nucleic acid molecules that encode candidate LEAD proteins, wherein:

each encoded candidate LEAD protein contains a single amino acid replacement;

each nucleic acid molecule in a set encodes the same candidate LEAD protein;

each candidate LEAD protein differs by one amino acid from the target protein or peptide, whereby the encoded candidate LEAD proteins in each set differ by one amino acid from the encoded candidate LEAD proteins in each of the other sets;

each set is separate from each and all other sets;

(d) individually introducing each set of nucleic acid molecules into host cells and expressing the encoded candidate LEAD proteins to produce sets of LEAD proteins, whereby:

each candidate LEAD protein in a set contains the same amino acid replacement;

the host cells comprise an addressable array such that each LEAD protein is expressed at a different locus in the array, and the identity of each candidate LEAD protein at each locus is known; and

(e) individually screening each set of encoded candidate LEAD proteins to identify one or more proteins that has an activity that differs from an activity of an unmodified target protein, wherein each such identified proteins is designated a LEAD mutant protein.

Claim 28 recites:

A method for generating proteins with a desired property or activity, comprising:

(a) identifying residues in a target protein *in silico* that are associated with the property, and designating the loci of such residues is-HIT target loci;

(b) preparing variant nucleic acid molecules encoding variant proteins, wherein each variant nucleic acid encodes a candidate LEAD mutant protein that differs by one replacement amino acid at one is-HIT target locus from the target protein, wherein:

the amino acid residues at each of the identified is-HIT target loci in the target protein is replaced with all of the non-native amino acids, or the amino acid residues at each of the identified is-HIT target loci in the target is replaced with a restricted subset of the remaining 19 non-native amino acids; and

a restricted subset is a group of selected amino acids selected to have a predetermined effect on protein activity.

(c) separately introducing the nucleic acid molecules encoding each candidate LEAD protein into hosts for expression thereof, and expressing the nucleic acid molecules encoding each variant protein to produce sets of candidate LEAD proteins, wherein:

each candidate LEAD protein in a set contains the same amino acid replacement;

each candidate LEAD protein contains a single amino acid replacement, and differs from the target protein by one amino acid replacement; and

each replacement is at the same locus; and

(d) individually screening each set of variant LEAD candidate proteins to identify any that have an activity or property that differs by a predetermined amount from the activity of the unmodified target protein, thereby identifying proteins that are LEADs.

Thus, in the instantly claimed methods residues for modification in a protein is/are identified *in silico*, variant polypeptides containing only a single amino acid change at an is-Hit locus separately produced by introducing encoding nucleic acid molecules into cells, which results in an addressable array, such that the identity of the encoded protein at each locus in the array is known, and each protein is separately screened to identify LEADs. Variant polypeptides containing all amino acid changes at each locus identified *in silico* or containing a restricted subset of amino acids are separately produced. Each variant polypeptide differs from the unmodified protein at only one amino acid. In dependent claims, variant proteins containing modifications from two or more different LEADs are produced and screened.

Each pending claim requires that each of the polypeptides that is generated differs from the original polypeptide by only one residue, and requires that each polypeptide is produced separately and screened separately.

Differences between the teachings of Ladner *et al.* and the instantly claimed methods

The method of Ladner *et al.* is a method for screening populations of **mixtures of proteins** encoded by **variegated** DNA, which by definition is a mixture of nucleic acid

molecules that results in a mixture of polypeptides. The encoded proteins contain one or more modifications; the variegated DNA is produced as a mixture of molecules that encode proteins that differ by one or **more** amino acids. In the method of Ladner *et al.* the modified proteins contain one or more modifications, the modified nucleic acids are prepared as a mixture and the encoded proteins screened from the mixture. The method of Ladner *et al.* does not include the steps of producing sets of polynucleotides that encode polypeptides that have one amino acid replaced compared to the wildtype (target) protein. Ladner *et al.* does not disclose individually introducing each set of nucleic acid molecules into host cells and expressing the encoded candidate LEAD proteins to produce sets of LEAD proteins, where the host cells are present in an addressable arrays, and where each LEAD protein in a set contains the same amino acid replacement. In the method of Ladner *et al.*. mixtures of nucleic acids that encode proteins modified at one or more loci are screened to identify proteins that bind to a target. In contrast, in the instantly claimed methods the polypeptides are produced such that they only differ at one locus from wild type. Each polypeptide is expressed and screened separately.

Ladner *et al.* teaches the generation of a "variegated" population of DNA molecules that encode modified polypeptides. This population constitutes a ***mixture of different DNA molecules that then are used to transform a cell culture***. The variegated DNA is a ***population of molecules that vary at a number of defined loci, typically at two to five bases,*** where the residues are varied through all 20 amino acids thus producing DNA molecules encoding a number of distinct potential target-binding proteins. The collection of molecules is expressed and screened. Hence the method of Ladner *et al.* differs from the instantly claimed methods in several respects. Ladner *et al.* does not prepare and screen individual mutants separately.

In particular, Ladner *et al.* teaches DNA binding proteins a process designated variegation of genes encoding known DNA binding proteins. The term variegated is defined in Ladner (see col. 12, lines 50-53):

> This application uses the term "variegated DNA" to refer to a population of molecules that have the same base sequence through most of their length, but that vary at a number of defined loci.

Hence the modified polypeptides are varied at number of loci; there is no suggestion for varying the polypeptides at one locus at a time.

Ladner continues at col. 17, lines 19-43:

> The fundamental principle of the invention is one of forced evolution. The efficiency of the forced evolution is greatly enhanced by careful choice of which residues are to be varied. The 3D structure of the potential DNA-binding domain and the 3D structure of the target DNA sequence are key determinants in this choice. First a set of residues that can either simultaneously contact the target DNA sequence or that can affect the orientation or flexibility of residues that can touch the target is identified. Then all or some of the codons encoding these residues are varied simultaneously to produce a variegated population of DNA. The variegated population of DNA is introduced into cells so that a variegated population of cells producing various potential-DBPs is obtained.
>
> The highly variegated population of cells containing genes encoding potential-DBPs is selected for cells containing genes that express proteins that bind to the target DNA sequence ("successful DNA-binding proteins"). After one or more rounds of such selection, one or more of the chosen genes are examined and sequenced. If desired, new loci of variation are chosen. The selected daughter genes of one generation then become the parental sequences for the next generation of variegated DNA (vgDNA).

In Ladner's method, which is for producing DNA binding proteins, residues to be varied are selected and then "***all or some***" of the residues are varied to produce a "variegated population of DNA" molecules. The application and claims state that the cell culture is transformed with a variegated gene encoding potential DNA-binding proteins or polypeptides, "where said cells collectively can express a plurality of different but sequence-related potential DNA-binding proteins or polypeptides. " Thus, in the Ladner *et al.* method, regions are identified, and ***one or more residues are modified in the DNA and populations (i.e. mixtures) of the varied (variegated DNA) are produced, then expressed and the populations are screened.***

In contrast, in the instantly claimed method, a region of the polypeptide to be varied is identified, and only one residue at a time is varied, and each modified polypeptide is individually expressed and tested to identify a LEAD(s). Hence Ladner *et al.* fails to teach several elements of the instantly claimed methods. In particular, Ladner *et al.* fails to disclose a method (claim 1 and dependents) that includes any or all of the steps of:

(1) producing a collection of sets of nucleic acid molecules that encode each of the candidate LEAD proteins, where:

each nucleic acid in a set encodes the same candidate LEAD protein that differs by one amino acid from the target protein or peptide, whereby the members of each set differ by one amino acid from the members of each of the other sets;

each set is separate from all other sets;

(2) producing a collection of sets of nucleic acid molecules that encode each of the candidate LEAD proteins, wherein:

each encoded candidate LEAD protein contains a single amino acid replacement;

each candidate LEAD protein contains a single amino acid replacement at an is-HIT locus;

each replacement is at the same is-HIT locus, whereby each candidate LEAD protein is the same;

each nucleic acid molecule in a set encodes the same candidate LEAD protein that differs by one amino acid from the target protein or peptide, whereby the members of each set differ by one amino acid from the members of each of the other sets;

each set is separate from each and all other sets;

(3) individually screening each set of encoded candidate LEAD proteins to identify one or more proteins that has an activity that differs from an activity an unmodified target protein, wherein each such identified proteins is designated a LEAD mutant protein.

Further Ladner *et al.* fails to teach a method that includes any or all of the steps of (claim 28 and dependents):

(1) preparing variant nucleic acid molecules encoding variant proteins, wherein each variant nucleic acid encodes a candidate LEAD mutant protein that differs by one replacement amino acid at one locus from the target protein at one is-HIT target residue;

(2) separately introducing the nucleic acid molecules encoding each candidate LEAD protein into hosts for expression thereof, and expressing the nucleic acid molecules encoding each variant protein to produce sets of LEAD proteins, wherein:

each candidate LEAD protein in a set contains the same amino acid replacement;

each candidate LEAD protein contains a single amino acid replacement; and

each replacement is at the same locus;

(3) individually screening each set of variant LEAD candidate proteins to identify any that have an activity or property that differs by a predetermined amount from the activity of the unmodified target protein, thereby identifying proteins that are LEADs.

Ladner *et al.* teaches ***none*** of steps (1)-(3) of either claim 1 or 28 nor any dependent claim. Ladner *et al.* teaches producing variegated DNA, which encodes mixtures of proteins with one or more amino acids changes. There is no teaching or suggestion in Ladner *et al.* for separately producing proteins with single amino acid changes, and screening them separately. Hence Ladner *et al.* prepare ***mixtures*** nucleic acid molecules and proteins screens the mixtures of proteins. Ladner *et al.* does not teach or suggest making single changes, expressing them and testing them separately. Ladner *et al.* does not produce addressable arrays, which are collections in which the identity of encoded proteins at loci (physical or otherwise identifiable

are known). The instantly claimed methods include *all* these elements. Stabach *et al.* ***does not*** cure these deficiencies.

Stabach *et al.*

Stabach *et al.* does not cure the deficiencies in the teachings of Ladner *et al.* Stabach *et al.* does not teach or suggest a method of directed evolution, nor any methods that include one or more of steps of making single changes, expressing them and testing them separately. Stabach *et al.* does not produce addressable arrays. Stabach *et al.* teaches identifying its proteins by sequencing.

Stabach *et al.* describes a study of substrate specificity of μ-calpain for the substrate αII spectrin by modifying the cleavage site of αII spectrin. Hence, Stabach *et al.* does not describe a method of directed evolution in which proteins are modified and screened for a predetermined property. Stabach *et al.* describes experiments designed to gain insight into the activity of μ-calpain. In the studies described in Stabach *et al.*, the penultimate residue, Val1175, flanking the major cleavage site in αII spectrin was replaced by "twenty different amino acids" to assess the effect on μ-calpain cleavage of αII spectrin. The spectrin variants were prepared by cloning human fetal αII spectrin. A clone containing bp 2531-4689 of the spectrin-encoding cDNA, was cloned into an inducible bacterial expression vector and site-directed mutagenesis using degenerate oligonucleotides encoding all 20 amino acids was used to replace the codon encoding Val1175 with each of the 20 possible amino acids, including with Val. Site directed mutagenesis of the codon encoding Val1175 was effected using a degenerate oligonucleotide containing all four degenerate nucleotides at this codon (see column 2, page 58, line 26 *et seq)*, **thereby generating a mixture of nucleic acid molecules encoding** variants. The goal was to produce 20 different variants, including the starting protein with Val1175, to produce 20 substrates upon which to test calpain to see which amino acids are important for cleavage specificity. Full-length clones were then regenerated in the mixture and the resulting clones were sequenced to identify those encoding variants with mutations. Sixteen different variants were generated by this method. The remaining three were prepared using three specific oligonucleotides. The effect of the mutations on cleavage by calpain were studied in an effort to understand calpain activity.

Hence Stabach *et al.* fails to teach or suggest a method of directed evolution, since Stabach *et al.* is modifying a substrate, not to change its activity, but to gain insight into calpain

activity. Furthermore, Stabach *et al.* produces mixtures of nucleic acid molecules which are then expressed in host cells, and clones are sequenced. Stabach *et al.* does not teach separately preparing variants; the variants are produced as mixtures; identification is effected by sequencing. Thus, Stabach *et al.* does not teach or suggest a method that includes any or all of the steps of making single changes, expressing them and testing them separately Stabach *et al.* does not produce addressable arrays, which are collections in which the identity of encoded proteins at loci (physical or otherwise identifiable are known). Stabach *et al.* fails to teach or suggest individually preparing the nucleic acids and introducing them into host cells to produce an addressable array. Stabach *et al.* employs degenerate oligonucleotides to produce a mixture of clones, which are then introduced into host cells. The mutants are identified by plating out clones, and sequencing them. Stabach *et al.* does not test known variants for a predetermined activity. Hence they are screened from a mixture. Further, the identity of each encoded protein is not known; no addressable array is produced. Thus, Stabach *et al.* fails to teach or suggest individually producing and introducing mutations into a protein one-by-one and individually producing the mutants in an addressable array. Stabach *et al.* does not teach screening any mutants, since the mutants are tested separately as part of a study of calpain activity. Hence, Stabach et al. does not cure the deficiencies in the teachings of Ladner et al.

Analysis

The combination of teachings of Ladner *et al.* with those of Stabach *et al.* does not result the claimed methods of either claim 1 or 28 nor any claim dependent thereon. The combination of teachings of Ladner *et al.* fails to teach or suggest at least the following elements of the instantly claimed methods: separately producing nucleic acid molecules, separately introducing them into host cells in addressable array, such that the identity of each nucleic acid molecule identity (and hence the encoded protein) is known; separately screening the proteins to identify those having a predetermined activity. Therefore, the Examiner **has failed to set forth a *prima facie*** case of obviousness.

Notwithstanding this the DECLARATION of Dr. Vega provided herewith demonstrates the power of the instantly claimed methods. The method is quite powerful and has been used successfully to identify a large number (i.e. thousands) of candidate LEAD proteins that are evolved to possess a predetermined property or activity with a minimal number, as few as one, of

amino acid changes. The resulting proteins retain the original activity. As described in the DECLARATION, the method has resulted in the identification of quite a few candidate LEAD protein molecules that possess a predetermined property or activity and retain an original activity of the unmodified polypeptide. The modified proteins have a minimal number of amino acid changes; the resulting polypeptides retain a desired therapeutic activity, but exhibit a change in a predetermined property. As described in the application and in the DECLARATION, the method is unbiased in that all possible intended variations are produced, there is no bias that results from any selective pressure in effecting the mutations, transducing or infecting host cells, growing the host cells and expressing the encoded protein. Since each is done individually, there is no selection amongst and between variants. The DECLARATION evidences the capacity of the instant method to identify mutant molecules with improved predetermined properties and/or activities. The DECLARATION also shows that the modified proteins retain desired activities/properties.

The DECLARATION describes that the instant method is an extremely powerful method that has been used to identify thousands of candidate LEAD molecules of a target polypeptide evolved to have a predetermined property, using a rational, unbiased approach in which each modified polypeptide is individually produced and screened. Using the methods as claimed in the instant application, LEAD polypeptides with an evolved predetermined property/activity are efficiently and effectively generated. As described in the DECLARATION, using the claimed methods, Nautilus Biotech, under the guidance of Manuel Vega and others at the company, has identified valuable candidate proteins for therapeutic use.

In the DECLARATION, data are provided evidencing that numerous LEAD polypeptides with a predetermined evolved property have been identified. The data show that, using the methods as claimed, hundreds of candidate LEAD polypeptides were identified and screened in each of IFN-alpha, IFN-beta, IFN-gamma, Growth Hormone and erythropoietin based on the predetermined property of protease resistance. The results show that the method as applied reliably and efficiently results in the generation of LEAD polypeptides exhibiting an evolved predetermined property, exemplified as increased protease resistance in the DECLARATION. The data also show that such polypeptides, with the increased protease resistance, have improved pharmacokinetic profiles following subcutaneous and per-oral administration.

The power of the method is evident when one considers that the method as claimed permits the discovery of therapeutic polypeptides, containing in many instances only a single amino acid, that are dramatically altered in the property evolved compared to the native polypeptide. For example, the DECLARATION provides data evidencing that a mutant IFN-α containing only a single amino acid mutation identified by the method based on the predetermined property of protease resistance, when administered subcutaneously or orally, retains anti-viral activity in the serum for a longer time period than the native polypeptide.

In the case of per-oral administration, the native polypeptide retains **no** detectable activity when administered; whereas, the IFN-α with a single amino acid change, identified by the methods herein, can be successfully administered orally. This is really astounding, and of enormous medical and economic value. Therefore, the results provided in the DECLARATION show that the methods as claimed have benefits that are not taught or suggested by any of the cited references. None of the cited references, singly or in any combination thereof, teaches or suggests a method as instantly claimed, in which target amino acids and replacement amino acids are identified *in silico*. None teaches or suggests a method in which mutations are introduced into nucleic acid molecules to individually and separately produce proteins containing only a single change from the original polypeptide. None, singly nor in any combination thereof, teaches that such combination of steps permits directed evolution of proteins to evolve a predetermined property/activity by changing only one or two or very few amino acids. Therefore, the Examiner has not established that any of the instant claims lack inventive step.

Claims 1,6, 15-18,28, 36-38, 52-55, and 81

Claims 1, 6, 15-18,28, 36-38, 52-55, and 81 are rejected under 35 U. S. C. 103(a) as being unpatentable over Ladner et al. in view of Stabach *et al.* as applied to claims 1, 4-11, 28-35, 51-52, and 80 above, in further view of Alam *et al.* (Journal of Biotechnology, volume 65, 1998, pages 183-1901) because Ladner *et al.* in view of Stabach *et al.* as applied to claims 1,4-l 1, 28-35, 51-52, and 80 above, does not teach increased resistance to proteolysis as a result of mutations. This deficiency is allegedly provided by Alam *et al.*, which allegedly render a portion human growth hormone that is not resistant to proteolysis, and mutate it render it resistant to proteolysis. The Examiner concludes that:

> [i]t would have been obvious to someone or ordinary skill in the art at the time of the instant invention to practice Ladner et al. in view of Stabach et

al. as applied to claims 1,4-l 1, 28-35, 51-52, and 80 above, in further view of Alam et al. to result in the instantly claimed invention because Alam et al. has the advantage of applying the site directed mutagenesis study to proteolysis for the purpose of better understanding of potential antibody production levels.

This rejection is respectfully traversed.

The claims

The claims are discussed above.

Ladner *et al.* and Stabach *et al.*,

Ladner *et al.* is discussed above. As discussed above, Ladner *et al.* fails to teach or suggest several elements of the claims of all of the claims, including modifying amino acids in a target region one-by-one, expressing the modified proteins individually them and screening them individually. As discussed above, Ladner *et al.* modifies one or more amino acids and produces mixtures of modified proteins that are screened. As discussed above, Stabach *et al.* fails to cure the deficiencies in the teachings of Ladner *et al.*

Alam *et al.*

Alam *et al.* teaches that the region from amino acids 134-150 hGH is cleaved by plasmin and thrombin and describes an hGH mutant (Arg to Asp at 134 and Thr to Pro at 135) that is resistant to proteolytic cleavage by thrombin. Alam *et al.* provides no teachings or suggestions regarding rational methods of protein evolution nor one in which amino acids are modified and the variant proteins tested one-by-one nor a method in which the variants are produced in addressable arrays such that the identity of each variant at a locus is known. Thus, Alam *et al.* fails to cure the deficiencies in the teachings of Ladner *et al.* in view of Stabach *et al.*

The combination of teachings of Ladner *et al.*, Stabach *et al.*, and Alam *et al.* does not result in the instantly claimed methods

The combination of teachings of the references fails to teach or suggest a method that includes the above noted steps, including modifying one amino acid at a time and expressing and screening each modified protein separately to identify LEADs that differ from the original protein at one locus. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

Claims 1-3

Claims 1-3 are rejected under 35 U. S. C. 103(a)as being unpatentable over Ladner et al. in view of Stabach as applied to claims 1, 4-11, 28-35, 51-52, and 80 above, further in view of Chiang et al. (Annual Reviews of Microbiology, 1999, volume 53, pages 129-1541) because Chang *et al.* teaches that "mutagenized bacterial strains are stored individually in arrays (usually in the wells of microtiter dishes).. ," thereby permitting "analysis to be performed simultaneously on a relatively large number of genes during an actual infection." The Examiner concludes that:

> [i] would have been obvious to someone or ordinary skill in the art at the time of the instant invention to practice Ladner et al. in view of Stabach as applied to claims1, 4-11, 28-35, 51-52, and 80 above, in further view of Chiang et al. to result in the instantly claimed invention because Chiang et al. has the advantage of applying the site directed mutagenesis study to the claimed condition of wells on a solid support for a more efficient analysis of gene expression.

This rejection respectfully is traversed.

Analysis

Ladner *et al.* and Stabach *et al.*,

Ladner *et al.* is discussed above. As discussed above, Ladner *et al.* fails to teach or suggest several elements of the claims of all of the claims, including modifying amino acids in a target region one-by-one, expressing the modified proteins individually them and screening them individually. As discussed above, Ladner *et al.* modifies one or more amino acids and produces mixtures of modified proteins that are screened. As discussed above, Stabach *et al.* fails to cure the deficiencies in the teachings of Ladner *et al.*

Chiang *et al.*

Chiang *et al.*, which is a review article describing various bacterial screening assay systems to identify the function of bacterial genes, is of little relevance to the instant claims. It does not teach any methods for modifying proteins and it fails to cure the deficiencies in the teachings of Ladner *et al.* in view of Stabach *et al.*

The combination of teachings of Ladner *et al.*, Stabach *et al* and Chiang *et al.* does not result in the instantly claimed methods

The combination of teachings of the references fails to teach or suggest a method that includes the above noted steps, including modifying one amino acid at a time and expressing and

screening each modified protein separately to case of obviousness. Therefore the Examiner has failed to set forth a *prima facie* case of obviousness.

Claims 1 and 78

Claims 1 and 79 are rejected under 35 U. S. C. §103(a)as being unpatentable over Ladner *et al.* in view of Stabach *et al.* as applied to claims 1,4-11, 28-35, 51-52, and 80, and further in view of Jones *et al.* (CABIOS, volume 8, 1992, pages 275-2821) because, Ladner et al. in view of Stabach *et al.*, does not teach PAM matrices, Jones *et al.* shows PAM matrices and states that it is the authors "hope that the matrices presented here will more clearly express the general nature of the underlying amino acid similarities." The Examiner concludes that:

> [i]t would have been obvious to someone or ordinary skill in the art at the time of the instant invention to practice Ladner et al. in view of Stabach as applied to claims 1, 4-l 1, 28-35, 51-52, and 80 above, in further view of Jones et al. to result in the instantly claimed invention because Jones et al. has the advantage of applying the site directed mutagenesis study to the claimed analysis condition of PAM matrices for the purpose of a clearer and more efficient understanding of the amino acid residues comprising the protein of interest.

This rejection respectfully is traversed.

This rejection respectfully is traversed.

Analysis

Ladner *et al.*

Ladner *et al.* is discussed above. As discussed above, Ladner *et al.* fails to teach or suggest several elements of the claims of all of the claims, including modifying amino acids in a target region one-by-one, expressing the modified proteins individually them and screening them individually. As discussed above, Ladner *et al.* modifies one or more amino acids and produces mixtures of modified proteins that are screened.

Chiang *et al.*

Chiang *et al.*, which is a review article describing various bacterial screening assay systems to identify the function of bacterial genes, is of little relevance to the instant claims. It does not teach any methods for modifying proteins and it fails to cure the deficiencies in the teachings of Ladner *et al.*

The combination of teachings of Ladner *et al.* and Chiang *et al.* does not result in the instantly claimed methods

The combination of teachings of the references fails to teach or suggest a method that includes the above noted steps, including modifying one amino acid at a time and expressing and screening each modified protein separately to case of obviousness. Therefore the Examiner has failed to set forth a *prima facie* case of obviousness.

Claims 1 and 79

Claims 1 and 79 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ladner *et al.* in view of Jones *et al.* ((1992) *CABIOS 8*:275-282), because Ladner *et al.* allegedly teaches all elements of claims 1 and 79, except for PAM matrices, which are taught by Jones *et al.* The Examiner concludes:

> it would have been obvious to someone or ordinary skill in the art at the time of the instant invention to practice Ladner *et al.* in view of Jones *et al.* to result in the instantly claimed invention because Jones et al. has the advantage of applying the site directed mutagenesis study of Ladner *et al.* to the claimed analysis condition of PAM matrices.

This rejection respectfully is traversed.

Analysis

As discussed above, **Ladner *et al.*** in view of Stabach *et al.* fails to teach or suggest several elements of the claims of all of the claims, including modifying amino acids in a target region one-by-one, expressing the modified proteins individually them and screening them individually. As discussed above, each of Ladner *et al.* and Stabach *et al.* teaches modification of one or more amino acids and production mixtures of modified proteins that are screened. These elements are not supplied by **Jones *et al.***, which describes PAM matrices.

Therefore, the combination of teachings of Ladner *et al.*, Stabach *et al.* and Jones *et al.*, singly or in any combination thereof, does not result in any of the instantly claimed methods. Thus, the Examiner has failed to set forth a *prima facie* case of obviousness.

Rebuttal to specific argument of the Examiner.

The Examiner applicant had argued that Ladner et al. is deficient in failing to teach modification at only a single residue. Ladner *et al.* does not teach that each polypeptide is expressed and screened separately. These deficiencies allegedly are cured by Stabach *et al.*

First, Applicant argue and argued that Ladner *et al.* fails to teach or suggest any all of the elements of a method using addressable arrays of variant polypeptides, individually preparing, expressing and screening variant polypeptides. **Second,** none of these deficiencies are cured by

the teachings of Stabach *et al.* Stabach *et al.* is merely a study of the activity of μ-calpain. Stabach *et al.* uses degenerate oligonucleotides to prepare **mixtures** of nucleic acid molecules and variant polypeptides. Stabach *et al.* does not teach or suggest addressable arrays nor separately expressing the encoded variant polypeptides. Stabach is virtually irrelevant to the instantly claimed methods. Accordingly, as discussed above, the combination of teachings of Ladner *et al.* and Stabach *et al.* cannot and does not result in any of the instantly claimed methods nor the results, evidenced by the DECLARATION and data in the application, achieved thereby.

Thus, the Examiner has failed to set forth a *prima facie* case of obviousness. The combination of teachings of the references does not result in the instantly claimed method. As discussed above, the combination of teachings in the references does not result in a method in which mutations are made one-by-one, expressed one-by-one such that the identity of the expressed protein is known, and tested one-by-one. Ladner *et al.* and Stabach *et al.* teach methods in which mixtures of oligonucleotides are used to produced mixtures of nucleic acid molecules that are introduced into host cells as mixtures. None of the cited references teaches or suggests anything that would have led the ordinarily skilled artisan to do that which applicant has done; none of the cited references teaches or suggests any reason to modify their methods. The prior art must provide a motivation whereby one of ordinary skill in the art would have been led to do that which the applicant has done. Stratoflex Inc. v. Aeroquip Corp., 713 F.2d 1530, 1535, 218 USPQ 871, 876 (Fed. Cir. 1983).

In addition, the mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggests the desirability of the modification. In re Fritch, 23 USPQ 1783 (Fed. Cir. 1992). There must be a reason why a person of ordinary skill in the art would have combined the elements as claimed. KSR v. Teleflex, Inc. 550 US _____ S.Ct. (2007). In this instance, the combination of teachings does not result in the instantly claimed methods, and there is no suggestion for modifying the methods of the prior to produce the instantly claimed methods, nor is there any reason provided in the cited art why one of ordinary skill in the art would have modified each step in the prior art methods to result in anything even resembling the instantly claimed methods.

* * *

In view of the above, entry of this amendment and examination of the application are respectfully requested.

Respectfully submitted,

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